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# Down to atomic-scale intracellular water dynamics

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## Abstract

Water constitutes the intracellular matrix in which biological molecules interact. Understanding its dynamic state is a main scientific challenge, which continues to provoke controversy after more than 50 years of study. We measured water dynamics in vivo in the cytoplasm of Escherichia coli by using neutron scattering and isotope labelling. Experimental timescales covered motions from pure water to interfacial water, on an atomic length scale. In contrast to the widespread opinion that water is 'tamed' by macromolecular confinement, the measurements established that water diffusion within the bacteria is similar to that of pure water at physiological temperature.



## Keywords

QENS, neutron scattering, Escherichia coli, water diffusion, in vivo measurement


## Disciplines

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**Down to atomic scale intracellular water dynamics**

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Keywords: QENS, Neutron scattering, *E. coli,* Water diffusion, *In vivo* measurement

**ABSTRACT**

Water constitutes the intracellular matrix in which biological molecules interact. Understanding its dynamic state is a major scientific challenge, which continues to provoke controversy after more than 50 years of study. We measured water dynamics *in vivo* in the cytoplasm of *E. coli* by neutron scattering and isotope labelling. Experimental time-scales covered motions from pure to interfacial water, on an atomic length-scale. Contrary to the widespread opinion that water is "tamed" by macromolecular confinement, the measurements established that water diffusion within the bacteria is pure-like at physiological temperature.

**INTRODUCTION**

More than 20 years ago, J. Clegg described the long-lasting challenge posed by gaining an understanding of water diffusion in the cell . Important progress has been made since but many questions still remain open. The apparent diffusion coefficient of water in biological tissues and in the brain has been measured by nuclear magnetic resonance (NMR). The measurements revealed that cell water diffusion on a micrometric scale is reduced by a factor of between 2 to 10 compared to pure water in aqueous media; the reduction could be explained partly by tortuosity effects, macromolecular crowding and confinement effects (reviewed by . The observation led to the emergence of diffusion magnetic resonance imaging (dMRI) to study the brain . In contrast, very little is known currently about cell water dynamics on a submicrometric scale. The question

can be addressed by using quasi-elastic neutron scattering (QENS) spectroscopy, which monitors the diffusive motions of hydrogens over a few Ångströms, on the pico- to nanosecond time domain. In this paper, we address the nature of intracellular water dynamics, using isotope labelling and incoherent neutron scattering, and present a direct measurement of water diffusion in live *E. coli* bacteria at physiological temperature, revealing the intrinsic physical properties of water in the cell interior on the atomic scale.

## RESULTS AND DISCUSSION

Neutron scattering dynamics measurements rely on incoherent scattering and can be performed on complex macromolecular systems such as living cells . Hydrogen $^{1}H$ nuclei have an incoherent neutron scattering signal at least forty times larger than that of deuterium ($^{2}H$) or any other nucleus or isotope in biological material. We performed QENS experiments on deuterated *E. coli* containing natural abundance $H_2O$. Deuterium labelling permitted the strong attenuation of the scattering signal from the deuterated cell components, revealing the water contribution. Intracellular water dominated the scattering signal at more than 93 % in the spectra (see Methods). The measurements were carried out on two neutron spectrometers, IN6 and IRIS, with complementary time-scales and atomic length-scale resolution (see Methods). The combined time-scale covered diffusive motions from pure to interfacial water. The IN6 spectrometer is well suited for the measurement of the rotational and translational contributions of pure water (Table

1). The IRIS spectrometer is optimal for the detection of reduced mobility interfacial water, as well as pure water translational motions.

In the case of simple exponential diffusion processes, the QENS spectra can be fitted mathematically by Lorentzian functions, as described in Methods. The dependence of the Lorentzian half-width at half-maximum (HWHM) on the wave-vector transfer modulus $Q$ contains information on the diffusion coefficient and other parameters of the process. *E. coli* water dynamics parameters extracted from IRIS data are reported in Fig 1 and Table 1. The QENS spectra were each fitted with one Lorentzian, which was attributed to translational motions of cell water (see Methods). Its HWHM, $\Gamma_T$ (Fig 1), was extracted and best fitted using a jump diffusion model , which describes diffusion between sites for the water protons with a mean residence time, $\tau_0$, at each site. $\Gamma_T$ follows:

$$\Gamma_T = \frac{D_T Q^2}{1 + D_T Q^2 \tau_0} \quad (1)$$

where $D_T$ represents the translational diffusion coefficient. We found translational diffusion coefficients that are very close to those of pure water at corresponding temperatures, with residence times about twice longer (Table 1). Furthermore, the $\Gamma_T$ behaviour at low $Q$ values indicated that *E. coli* water does not act as if it were confined (Fig 1). IRIS results, although

obtained on a time-scale optimal for the detection of reduced mobility interfacial water, revealed therefore an *E. coli* water translational contribution that was neither confined nor significantly slowed down compared to pure water.

The IN6 spectrometer is well suited for the measurement of the rotational and translational contributions of pure water. The IN6 QENS spectra were fitted with two Lorentzian functions (see Methods). The translational and rotational motions of cell water were extracted respectively from the narrow and broad Lorentzians (Fig 2). Identical parameters for translational diffusion, as those found on IRIS, were extracted from IN6 data (Table 1). In addition, the rotational contribution to the motions emerged (Fig 3). The HWHM of the broad Lorentzian shows a *Q*-independent profile, as expected for rotational motions . Rotational correlation times are close to the values extracted for the buffer under the same conditions and of the same order as the values measured for pure water by QENS and NMR . Protein hydration water rotational relaxation times are slowed down by a factor of between 5 to 10 compared with pure water . We concluded, therefore, that *E. coli* water diffusive motions measured over atomic distances are dominated by a pure-like cell water component at physiological temperature.

The study on *E. coli* contributes to direct evidence, which dismantles the idea that the cell somehow 'tames' water and renders it very different from pure water . In combination with previous work, it confirms that hydration degree plays a crucial role in the dynamic state of water in confined geometries. Studies on water dynamics in deuterated C-phycocyanin have found

increasing water mobility already when hydration coverage reached one water layer . Reduced cellular water mobility on an atomic scale has so far been observed only at cryogenic temperatures , in low hydrated *Artemia* shrimp cysts or in extreme halophiles . *E. coli* contains about 2.6 g/g macromolecule of cellular water, in which the first hydration layer accounts for about 0.5 g/g macromolecule . On average, cell water would therefore correspond to about five water layers around macromolecular surfaces. We note, however, that the residence time was found to be higher by a factor of two than that of pure water. This average may reflect the longer times spent by the water molecules in the hydration shells. Once released, however, exchange with external layers would take place with the diffusion rate of pure-like water.

The concept derived from indirect experimental observations that the cell interior is a gel or colloidal-like structure, in which confined water is interfacial with properties that are significantly different from those of pure water, has influenced current thought in parts of the biology community. A 2006 book edited by Pollack et al. states already in the preface '... practically all cell water is interfacial' . The claim, however, remains controversial as reviewed by . Our data derive from direct measurement of water properties *in situ* and *in vivo* on a wide range of time-scales, and refute this concept. They show, on the contrary, that water forms a network of communication in the crowded cell interior that is as fluid as pure water. Previous work performed by Tehei and coworkers had already established that *E. coli* did not contain a water component that is slower than interfacial hydration water by two orders of magnitude, as was

observed in the special case of Dead Sea archaea . The origin of the very slow water component in these extreme halophiles is still under investigation; it is unrelated to molecular crowding or the high salt solution and was interpreted as due to specific interactions involving macromolecules, water and salt ions, which are yet to be defined.

Our data showed that water dynamics is different on micrometric and atomic scales. Micrometric scale measurements probe cell water diffusion over the entire cell length, along which water can encounter a large number of obstacles (reviewed by . Water in the extracellular matrix may also contribute appreciably in the micrometric scale measurements, depending on the sample state and experimental design. Differences could emerge, finally, between different cell types, such as prokaryotic and eukaryotic cells, for example, arising from the presence of cytoskeleton and nucleus. The value of the viscosity depends also strongly on the length-scale that is probed, as reported in previous work . The results obtained for intracellular water dynamics on an atomic scale suggest that atomic scale viscosity in the cell cytoplasm is close to the viscosity measured in pure water (viscosity is often calculated using diffusion parameters, according to the Stokes Einstein relation).

It is important finally to emphasize the fundamental biological relevance of the results. It is very likely that the remarkable properties of bulk water are essential for cellular viability, in particular for functional macromolecular folding, stabilisation and activity, transport, membrane formation and protein insertion into membranes. Whenever interfacial water has been obtained in the

presence of biological macromolecules, it has always been after these were already synthesised, correctly folded, stabilised, and the bulk water subsequently dried or frozen out. Our data show that the water in-between the macromolecules in the *in vivo* intracellular environment has essentially pure-like properties and can therefore participate as such in all the interactions described above.

To conclude, borrowing from the poetic terms that have been used to describe cell water wrongly, our paper shows that water is not 'tamed' by the cell environment nor is "life ... water dancing to the tune of macromolecules" (Albert Szent-Gyorgyi cited in ) but quite vice versa.

## METHODS

### Sample

Deuterated *E. coli* (BLE21(DE3) strain) were cultivated at 37°C to the stationary phase, with an optical density value of 1.5, in deuterated Enfors minimum growth medium with deuterated glycerol (d8) as the carbon source. Cells were pelleted by centrifugation at 5000 rpm in a Beckman centrifuge (JLA10500 rotor) for 20 minutes at 4°C. The supernatant was discarded and the cells were washed twice with 200 ml of $H_2O$ or $D_2O$ buffer solution (150 mM NaCl, 5 mM KCl, 10 mM Tris-HCl (respectively Tris-DCl) pH 6.6). The cells were pelleted via 20 minutes centrifugation, transferred to aluminium sample holders (4 x 3 x 0.02 $cm^3$), and sealed with an indium 'o' ring for the neutron measurements. After the experiments, a small amount of the pellet

was resuspended in the buffer and layered on Petri dishes after several dilutions steps. The number of colonies was compared to that obtained for cells from the fresh culture, and found to be similar, which indicated that most of the cells remained intact and viable after the total beamtime exposure. The rest of the cell pellet was dried in an oven at 80°C for a few days; the quantity of extracellular water was evaluated taking into account that the proportion of intracellular water is approximatively 72 % of *E. coli* total cell weight . Extracellular water was found to be less than 7 % of the total water.

**Neutron measurements**

The full-width at half-maximum (FWHM) energy resolution of a neutron spectrometer defines the upper limit of the motions time-scale. IN6 (FWHM = 90 μeV) at the Institut Laue Langevin (ILL, Grenoble, see www.ill.fr for further information) is suitable for the measurement of motions with characteristic times, $\tau$ < 15 ps, whereas IRIS (FWHM = 17 μeV), at ISIS, Chilton (http://www.isis.rl.ac.uk) can resolve slower motions, with $\tau$ < 75 ps. The scattering was measured over a wave-vector range of 0.5 $Å^{-1} < Q < 1.7$ $Å^{-1}$, associated with atomic length-scale, for each spectrometer. A vanadium sample (a purely incoherent elastic scatterer) was measured to define the instrument resolution and correct for detector efficiency. IRIS spectra were corrected for detector efficiency, sample container and *E. coli* macromolecular contribution (see following paragraph), normalized, grouped, and transferred into energy transfer spectra using the IRIS data

reduction program, Ionian, from the MODES package . IN6 spectra were corrected in the same way, normalized, and the scattering function, S ($\mathbf{Q},\omega$), where $\omega$ is the energy transfer, was extracted by using the LAMP data reduction routines (LAMP). The sample transmissions were > 90 % on both instruments and multiple scattering was neglected.

**Data collection**

Cell water dynamics was studied by measuring two samples: deuterated cell pellets resuspended in $H_2O$ buffer, on the one hand, and deuterated cells resuspended in $D_2O$ buffer, on the other. A subtraction of the cell spectra measured in $D_2O$, from the cell spectra measured in $H_2O$, scaled by the sample mass, provided a good approximation to the scattering signal from the water present in the samples. We recall that extracellular water was found to be less than 7 % of the total water. The intracellular water, therefore, dominated the scattering signal by more than 93 % in the spectra corrected for macromolecular contribution as explained above. A sample of the $H_2O$ buffer alone was measured on each spectrometer as well, and used as a reference for interpreting the data.

**Data analysis**

In the case of translational diffusion and rotational motions of water, the calculated scattering function, $S_{calc}(\mathbf{Q},\omega)$, can be expressed phenomenologically as follows :

$$S_{calc}(\mathbf{Q},\omega) = e^{-\langle x^2\rangle Q^2/3}\left[A_{1,T}(\mathbf{Q})L(\Gamma_T,\omega) \otimes \left(A_{0,R}(\mathbf{Q})\delta(\omega) + A_{1,R}(\mathbf{Q})L(\Gamma_R,\omega)\right)\right] \quad (2)$$

where $e^{-\langle x^2\rangle Q^2/3}$ is a Debye-Waller (DW) factor, which accounts for vibrational modes; the DW factor is simply a scaling factor in $\omega$-space that does not modify the shape of the quasi-elastic scattering function. The quasielastic component arises from the convolution of the translational and rotational scattering functions, which are described by Lorentzian functions:

$$L(\Gamma_i,\omega) = \frac{1}{\pi}\frac{\Gamma_i(\mathbf{Q})}{\Gamma_i(\mathbf{Q})^2 + \omega^2} \quad (3)$$

where $\Gamma_i$ is the half-width at half-maximum (HWHM) of the Lorentzian peak. Eq. 2 simplifies as follows:

$$S_{calc}(\mathbf{Q},\omega) = e^{-\langle x^2\rangle Q^2/3}\left[A_1(\mathbf{Q})L(\Gamma_T,\omega) + A_2(\mathbf{Q})L(\Gamma_R + \Gamma_T,\omega)\right] \quad (4)$$

The experimental scattering function, $S_{exp}(\mathbf{Q},\omega)$, is obtained by convoluting $S_{calc}(\mathbf{Q},\omega)$ with the energy resolution of the spectrometer, $S_{res}(\mathbf{Q},\omega)$, determined by the vanadium sample:

$$S_{exp}(\mathbf{Q},\omega) = e^{-\hbar\omega/2k_BT}[\left(e^{-<x^2>Q^2/3}A_0(\mathbf{Q})\delta(\omega) + S_{calc}(\mathbf{Q},\omega)\right) \otimes S_{res}(\mathbf{Q},\omega)] + B_0 \quad (5)$$

where $e^{-\hbar\omega/2k_BT}$ is a detailed balance factor, $A_0$ is the elastic incoherent structure factor (EISF) arising from the slow motions that are not resolved by the instrumental resolution, and $B_0$ an inelastic background due to vibrational modes of lowest energy, which recalls the "lattice phonons" in crystals .

On IRIS, the rotational motions of cellular water contribute to the scattering signal only as a flat background. The IRIS spectra were fitted well according to Eq. 4, 5 by a single Lorentzian, arising from the translational diffusion of cell water. The fits were performed over the energy transfer range -0.2 to +0.5 meV by using the IRIS Bayesian fitting program *QL function,* from the MODES package .

The IN6 spectra were fitted according to Eq. 4, 5 by two Lorentzians, over the energy transfer range -1.5 meV to +1.5 meV, by using the QENS_FIT routine present in the LAMP package (LAMP). The narrow Lorentzian arises from the translational diffusion of cell water. The broad Lorentzian arises mainly from the rotational contribution, which was broadened, however, by the translational contribution as described in Eq. 4. The rotational correlation times, $\tau_{cor,R} = 1/\Gamma_R$, were extracted from the broad Lorentzian after correction for the broadening arising from the translational contribution.

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## LEGENDS TO FIGURES

**Fig 1.** IRIS data. Half-width at half-maximum of the translational Lorentzian, $\Gamma_T$, as a function of $Q^2$, at 281 K (filled black squares) and 301 K (filled black triangles). $\Gamma_T$ was best fitted (solid lines) using a jump diffusion model (Eq. 1). We found $D_T = (1.53 \pm 0.05) \times 10^{-5}$ cm$^2$/s, $\tau_0 = 2.63 \pm 0.11$ ps at 281 K and $D_T = (2.39 \pm 0.05) \times 10^{-5}$ cm$^2$/s, $\tau_0 = 2.16 \pm 0.05$ ps at 301 K.

**Fig 2.** IN6 QENS data and applied fits using Eq. 4, 5 for *E. coli* cell water. Quasi-elastic spectra at $Q = 1.5$ Å$^{-1}$, at T = 281 K (**a**) and T = 301 K (**b**). The data are indicated with the associated error bars. The bold and dotted lines represent the total fitted curve and the elastic peak, respectively. The narrow Lorentzian (dash line) was attributed to translational motions and the

broad component (dash-dot line) was attributed to rotational motions of cell water, broadened by the translational contribution (see Methods).

**Fig 3.** IN6 data. Half-width at half-maximum of the broad Lorentzian, $\Gamma_R + \Gamma_T$, as a function of $Q^2$, at 281 K (filled black squares) and 301 K (filled black triangles). The rotational correlation times, $\tau_{cor,R} = 1/\Gamma_R$, were extracted from the broad Lorentzian after correction for the broadening arising from the translational contribution, and found to be 1.96 ± 0.01 ps at 281 K, and 1.54 ± 0.01 ps at 301 K.

## TABLES

| Spectrometer | Origin of water | $D_T$ | $\tau_0$ | $\tau_{cor,R}$ | References |
|---|---|---|---|---|---|
| (FWHM, μeV) | (T, K) | ($10^{-5}$ $cm^2.s^{-1}$) | ps | ps | |
| IRIS (17) | *E. coli* cells (281) | **1.53** ± 0.05 | **2.63** ± 0.11 | | This work |
| IN6 (90) | *E. coli* cells (281) | **1.78** ± 0.08 | **2.95** ± 0.11 | **1.96** ± 0.07 | This work |
| IRIS (17) & IN6 (90)* | *E. coli* buffer (281) | **1.68** ± 0.04 | **1.48** ± 0.07 | **1.89** ± 0.07 | This work |
| IN6 (100) | Pure (285) | **1.6** | **1.66** | | |
| MIBEMOL (28; 96) | Pure (298) | **2.3** | **1.1** | **3.3** | |
| IRIS (17) | *E. coli* cells (301) | **2.39** ± 0.05 | **2.16** ± 0.05 | | This work |
| IN6 (90) | *E. coli* cells (301) | **2.94** ± 0.40 | **2.28** ± 0.27 | **1.54** ± 0.08 | This work |
| IRIS (17) & IN6 (90)* | *E. coli* buffer (301) | **2.72** ± 0.17 | **1.04** ± 0.15 | **1.47** ± 0.08 | This work |

**Table I.** Translational and rotational parameters of water in the pure state, in the cell and in the buffer. **E. coli* buffer was measured both on IRIS and IN6. The translational parameters were identical on the two spectrometers. The rotational correlation times were extracted from the IN6 data.

**FIGURES**

**Fig 1**

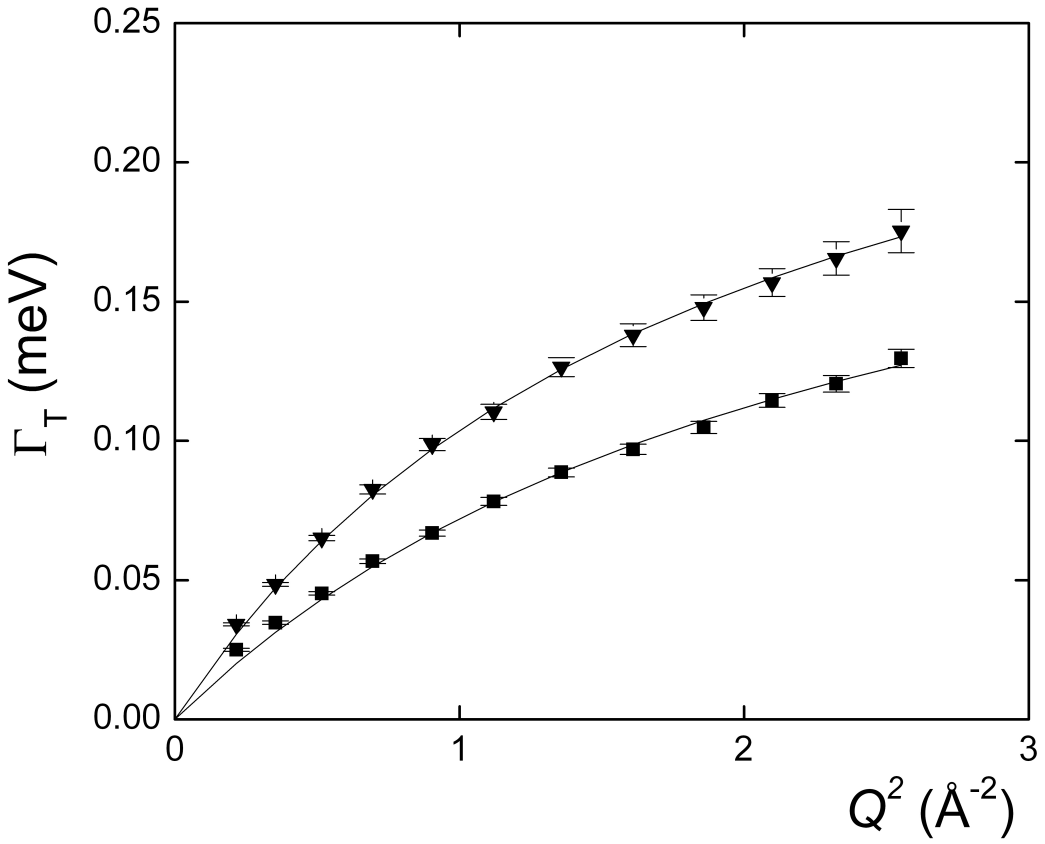

**Fig 2**

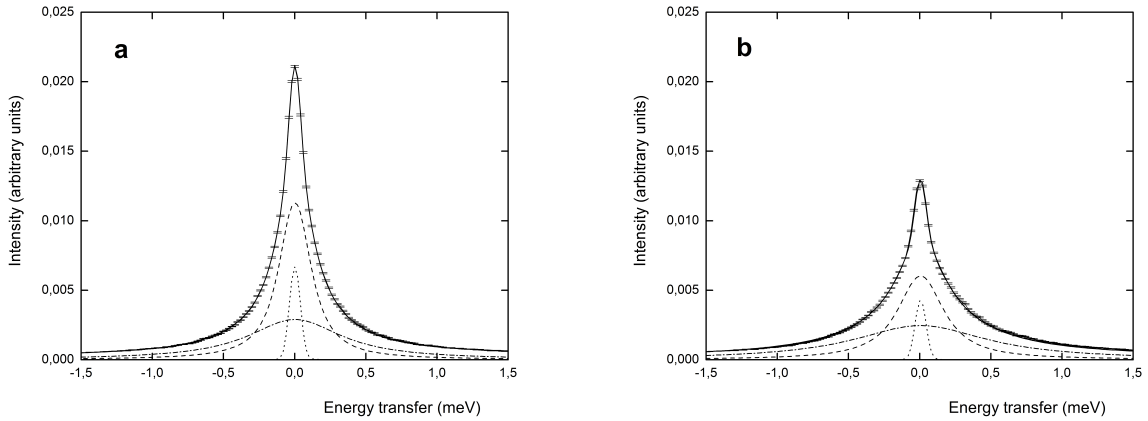
a
b
Intensity (arbitrary units)
Energy transfer (meV)
0,025
0,020
0,015
0,010
0,005
0,000
-1,5
-1,0
-0,5
0,0
0,5
1,0
1,5

**Fig 3**